

Biochemical Response of Cocoa (*Theobroma cacao* L.) Genotypes to Water Deficit Stress Condition

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ABSTRACT

Climate change and water shortages are the main challenges to cocoa production worldwide. Raising concerns about climate change and changing rainfall patterns necessitates screening for drought tolerance in cocoa genotypes in order to achieve long-term sustainability of production. The present study was conducted to evaluate the biochemical responses of six cocoa hybrid genotypes under two water deficit conditions, i.e., 100% FC and 40% FC. Biochemical parameters such as proline, nitrate reductase, superoxide dismutase (SOD), and glycine betaine (GB) were estimated. The biochemical parameters, proline, superoxide dismutase (SOD) and glycine betaine (GB) content were increased under drought stress condition

at 40% FC. However, nitrate reductase recorded a decline in its activity under drought stress in all the genotypes. The results revealed that all the parameters were significantly differed in both the water deficit conditions and among the cocoa genotypes. The results of the study concluded that, there is a role or involvement of these parameters in imparting drought tolerance to cocoa and these biochemical parameters can be used as a promising biomarkers or indicators for identifying stress tolerance in cocoa.

Keywords Cocoa, Genotypes, Proline, Nitrate reductase, Water deficit stress.

INTRODUCTION

Cocoa is a high-demanding industrial crop which has significant importance in the confectionary industry because its beans are the main ingredient for chocolate production. It also serves as a main source of bioactive compounds used in the cosmetic and pharmaceutical industries (Zambrano *et al.* 2021). It is native to the tropical humid rain forest of South America, requires an annual rainfall of 1500–3000 mm. About 73% of the world's production of cocoa is obtained from African countries, followed by Asian and Latin American countries (ICCO 2017). Climate change and water scarcity pose a serious threat to cocoa production worldwide.

Globally, drought is one of the primary causes of plant yield reductions of more than 50%. Plants show multiple responses to drought, and it mainly

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affects metabolic and physiological processes in plants (Efeoglu *et al.* 2009). Normally, plant growth is affected by drought stress through altering various physiological and biochemical mechanisms. Apart from physiological and biochemical parameters, the antioxidant responses have also been considered as an effective bio-markers for identifying drought tolerance in plants (Juby *et al.* 2021).

Plant responses to drought stress mainly involve the production and accumulation of osmolytes. Proline is the most common osmolyte produced in plants under water deficit stress condition. Accumulation and metabolism of proline is one of the major adaptive mechanisms linked to abiotic stress avoidance in crops (Dzandu *et al.* 2021). Glycine betaine is another compatible solute accumulate under water deficit conditions. This is a protein stabilizing osmoregulator which safeguard protein denaturation and deactivation thus reduce lipid oxidation and membrane damage under stress (Papageorgiou and Murata 1995).

Nitrate reductase is a key enzyme which has a significant role in N uptake and assimilation in plants. Nitrate reductase activity is related to the physiological and metabolic state of plants and can be utilized as a biomarker of drought stress tolerance (Janani *et al.* 2019).

Under drought stress, there is an increase in the synthesis and accumulation of reactive oxygen species (ROS), which results in oxidative stress and the activation of antioxidant systems in plants (Plazas *et al.* 2019). Superoxide dismutase (SOD) is one among the ROS scavenging enzymes, which minimizes the damage caused by oxidative stress under drought stress in plants (Toscano *et al.* 2016). These biochemical parameters can be used as a promising bio-markers in screening of genotypes for drought tolerance in cocoa.

Better understanding and involvement of these biochemical parameters in the adaptive mechanism of cocoa in water stress tolerance is need of the hour for germplasm evaluation and creating new varieties. Hence, this study was carried out to investigate the effect of water deficit stress on biochemical param-

eters of six cocoa genotypes.

MATERIALS AND METHODS

The research work was carried out during 2020–2022 at Cocoa Research Center, Kerala Agricultural University, Thrissur with an altitude (10° 32' N, 76° 17' E) in a greenhouse. Six cocoa genotypes namely, P.IV 19.9, P.II 12.11, P.IV 59.8, VSD.I 11.11, VSD.I 3.4 and VSD.I 29.9 were budded on six month old root-stock of seedling raised from polyclonal gardens. The genotype, GIV 18.5 (CCRP 5) (progeny of pods from Nileshwar) which is highly susceptible identified by (Binimol 2005) is used as a check in the experiment. Two irrigation regimes i.e., 100% field capacity and 40% field capacity were imposed as treatments on six month old budded plants using gravimetric method (Souza *et al.* 2000). Plants were maintained on 40% FC for one week. The percentage of leaves retained were recorded after one week of stress imposition. Morphological classification of genotypes as highly tolerant (> 70%), tolerant (40.1-70%), susceptible (10.1-40%) and highly susceptible (0-10%) were done according to the score chart by Juby *et al.* (2021). The percentage of leaves retained was calculated by recording the total number of leaves and the leaves retained after one week of stress imposition.

Assessment of biochemical parameters

Biochemical parameters were assessed at both the irrigation regimes of 100%FC and 40%FC. Assessment of Proline ($\mu\text{g/g}$) from six genotypes of cocoa along with check variety were done using method proposed by Bates *et al.* (1973). A sample of 0.5 g fresh leaf material in 3% sulpho salicylic acid was used for extraction and estimation was done with ninhydrin reagent according to above reported method. Absorbance value or optical density was recorded at 520 nm wavelength in spectrophotometer. A calibration curve was used for proline concentration to determine factor and proline content was calculated using following formula.

$$\mu\text{moles/ g tissue of proline} = \frac{\mu\text{g of proline} \times \text{ml toluene} \times 5}{115.5 \times \text{g sample}}$$

Nitrate reductase activity was estimated using method reported by Evans and Nason (1953). One gram leaf

sample were taken and suspended in 5 ml reaction mixture consisting of 5% propanol along with 0.02% potassium nitrate dissolved in 0.1 M potassium phosphate buffer. After 2 hr of incubation at room temperature, 0.4 ml of the reaction mixture was taken from the sample and 0.2 ml of 1% sulphanilamide and 0.2 ml of 0.2% N-naphthyl ethylene diamene dihydrochloride were added. After 20 minutes, 4 ml of distilled water was added. Absorbance value or optical density was measured at 570 nm wavelength in spectrophotometer.

The parameter, glycine betaine (GB) was estimated using method reported by Grieve and Grattam (1983). Finely grounded dried cocoa leaf sample of 500 mg weight added in 20 ml of distilled water and it was mechanically shaken for 24 hrs at room temperature. After 24 hrs these samples were filtered through muslin cloth by adding 20ml of distilled water to the filtrate for extraction. Aliquots (0.5 ml each filtrate) after dilution with 2N H₂SO₄ was cooled in ice water for 1 hr, and 0.2 ml of cold potassium tri iodide solution was added to it and these tubes were gently vortexed, stored at 0°C for 15 minutes. The supernatant formed was removed by centrifugation process and then per-iodide crystals were dissolved in 9 ml of 1,2 – Dichloroethane. For solubilization of crystals vigorous vortexing of tubes were done. After 2.5 hrs of incubation time, the absorbance was measured at 365 nm in a spectrophotometer. Glycine betaine content of sample was calculated using standard curve from graph using formula and was expressed in µmol/g dry weight basis.

Determination of Super oxide dismutase (SOD) activity was done through the method reported by Dhindsa *et al.* (1981). About 0.2 g of leaf sample was ground with 2 ml extraction buffer for extracting enzyme source by centrifugation process at 10,000 rpm for ten minutes at 4°C. The supernatant obtained after centrifugation can be used within 12 hrs and the supernatant was mixed to 3 ml reaction mixture and then 0.1 ml riboflavin was added to it. The reaction mixture tubes were kept under 15W fluorescent lamps for 15 minutes then it is placed in dark condition to stop the photochemical reaction. Absorbance was taken at 560 nm in spectrophotometer. Enzyme activity estimated using the following formula. It is expressed as units/mg of protein.

$$\text{Unit (of enzyme)} = \frac{\text{Blank} - \text{Sample}}{\text{Blank}/2}$$

Statistical analysis and correlation studies

All the collected data were subjected to analysis of variance (ANOVA) and significance at level of 0.05 probability to differentiate different treatment means. To ascertain the degree of relationship between characters and the percentage of leaves retained, correlation study was performed

RESULTS AND DISCUSSION

After one week of stress imposition, the percentage of leaves retained was calculated using visual observation and these selected six cocoa genotypes and check variety were categorized as highly tolerant, tolerant, susceptible and highly susceptible according to the standardized score chart given in the Table 1. which depicts the morphological classification of cocoa genotypes to drought stress. Estimation of different biochemical parameters under both 100% and 40% field capacity levels are depicted in Table 2. These estimations will contribute to involvement and reliability of these parameters in the drought adaptive mechanism in cocoa genotypes.

Effect of water deficit stress on proline content

Proline is the major and common osmolyte accumulated in plants subjected to stress. It function as both osmoregulator and radical scavenger at the onset of drought stress. All the genotypes evaluated in this study recorded significant differences for proline content for both the field capacity level (Table 2).

Table 1. Morphological classification of cocoa genotypes towards drought stress.

Sl. No.	Genotype	Percentage of leaves retained (%)	Reaction to drought stress
1	P.IV 19.9	81.81	Highly tolerant
2	P.II 12.11	20.83	Susceptible
3	P.IV 59.8	66.66	Tolerant
4	VSD.I 11.11	47.06	Tolerant
5	VSD.I 3.4	52.94	Tolerant
6	VSD.I 29.9	66.67	Tolerant
7	CCRP 5 check	9.09	Highly susceptible

Generally under well-watered condition i.e., 100% field capacity level, all the genotypes recorded lower proline content than 40% FC of water stress condition. This implies that elevation in production and accumulation of proline is seen under drought stress and this helps the plants to maintain the osmoregulation and survive under water stress. P.IV 19.9 (653.50 $\mu\text{g/g}$) which is classified as highly drought tolerant recorded higher proline content while check variety (194.85 $\mu\text{g/g}$) which is highly susceptible recorded the least when subjected to water stress. Lower amount of proline content was estimated in P.II 12.11 (208.93 $\mu\text{g/g}$) which is classified as susceptible compared to all other genotypes when subjected to water stress. Proline content in leaves of P.IV 19.9 (653.50 $\mu\text{g/g}$) elevated to more than 4 fold level under water deficit condition compared to well watered condition. Under water deficit condition when all the genotypes compared with check variety proline content ranged from 194.85 $\mu\text{g/g}$ to 653.50 $\mu\text{g/g}$. In a study Janani *et al.* (2019) also observed 5-6 times higher proline content in cocoa clones under water stress condition. Higher proline accumulation has been correlated with stress tolerance in plants (Carvalho *et al.* 2019). A study reported by More *et al.* (2019) observed that drought tolerant genotypes in taro recorded 2-3 fold higher proline accumulation than susceptible one under water deficit stress compared to control. This is an adaptive mechanism to withstand deleterious effect of water stress in plants. So this can be advocated as a reliable parameter to select drought stress tolerance in plants. The results in this experiment are in agreement with Mafakheri *et al.* (2010), Datta *et al.* (2016), Carvalho *et al.* (2019)

and Savaliya *et al.* (2019). Higher accumulation of proline under drought stress conditions have been found in many crops, including, mung bean (Dutta *et al.* 2016), maize (Majeed *et al.* 2020, Voronin *et al.* 2019), cotton (Zhang *et al.* 2021), canola (Din *et al.* 2011 and Li *et al.* 2017) and cacao (Bae *et al.* 2009; Dzandu *et al.* 2021, Janani *et al.* 2019 and Juby *et al.* 2021).

Effect of water deficit stress on nitrate reductase activity

Nitrate reductase enzyme has an important role in nitrogen assimilation and nitrate uptake in plants. Results pertaining to Nitrate reductase activity revealed that there is significant variation among all the genotypes at both the irrigation regimes (Table 2). Reduction in Nitrate reductase activity was recorded in all genotypes studied as a result of water stress. Higher activity was recorded in 100% field capacity compared to stress condition. Maximum Nitrate reductase activity of about 11.90 mmol nitrate/g/hr was recorded in P.IV 19.9 which is classified as a highly drought tolerant genotype followed by VSD.I 29.9 (7.88 mmol nitrate/g/hr), P.IV 59.8 (7.31 mmol nitrate/g/hr), VSD. I 3.4 (7.15 mmol nitrate/g/hr) and VSD.I 11.11 (6.99 mmol nitrate/g/hr) which are of drought tolerant group under stress condition. Compared to all genotypes check variety (4.08 mmol nitrate/g/hr) recorded lowest activity under stress condition. Imbalance in nitrate flux under water stress, leads to decline in protein synthesis which results in a decline in the synthesis of Nitrate reductase activity (Costa *et al.* 2008). In this study, when highly drought

Table 2. Effect of water deficit condition of 100% FC and 40% FC for of cocoa genotypes for biochemical parameters.

Sl.No.	Genotype name	Proline content ($\mu\text{g/g}$)		NRA (mmol nitrate/g/hr)		Glycine betaine ($\mu\text{mol/g}$)	
		100% FC	40% FC	100% FC	40% FC	100% FC	40% FC
1	P.IV 19.9	136.46	653.50	14.30	11.09	4.41	8.92
2	P.II 12.11	69.31	208.93	12.95	4.14	2.61	5.22
3	P.IV 59.8	82.04	436.57	14.26	7.31	4.23	6.64
4	VSD.I 11.11	76.80	483.15	12.27	6.99	3.21	5.58
5	VSD. I 3.4	93.21	461.01	14.02	7.15	3.46	7.85
6	VSD.I 29.9	94.35	516.17	13.29	7.88	3.91	6.77
7	CCRP 5 check	65.27	194.85	11.66	4.08	2.24	4.86
CD (5%)		8.231	29.33	8.231	1.297	0.842	0.407
SE (m)		2.688	9.578	2.688	0.424	0.275	0.133
CV (%)		5.277	3.931	5.277	5.537	6.855	6.705

tolerant and tolerant genotypes subjected to drought stress they had a substantial amount of enzyme while susceptible and check variety had very low amount present in them. Higher amount of Nitrate reductase activity attributed to their drought tolerance nature and these genotypes were able to maintain Nitrate reductase activity even at low water level. This implies the role of NRA activity in drought stress tolerance. According to Foyer *et al.* (1998), low nitrate absorption and availability during water stress resulted in a decline in nitrate reductase activity in maize. Finding in this investigations are in agreement with Ghosh *et al.* (2000) and Xu and Yu (2006).

Effect of drought stress on glycine betaine (GB)

Another important biochemical parameter which has a significant role in drought stress tolerance is glycine betaine. Accumulation of compatible solutes is one of the major adaptive mechanism in plants to deal with stress situation. In the present investigation, all the genotypes recorded significant variation for glycine betaine content under both the field capacity level (Table 2). Glycine betaine content increased under 40% field capacity in all the genotypes including check variety. P.IV 19.9 (8.92 $\mu\text{mol/g}$) classified as a highly drought tolerant genotype recorded higher GB content followed by VSD. I 3.4 (7.85 $\mu\text{mol/g}$), VSD.I 29.9 (6.77 $\mu\text{mol/g}$), P.IV 59.8 (6.64 $\mu\text{mol/g}$) and VSD.I 11.11 (5.58 $\mu\text{mol/g}$) which are group of tolerant genotypes under water stress condition. While check variety (4.86 $\mu\text{mol/g}$) which is highly susceptible recorded the least GB content. Glycine betaine functions as an osmoregulator and maintains the membrane integrity of plants to cope with the stress condition (Juby *et al.* 2021). Rhodes and Hanson (1993), Yang and Miao (2010) and Juby *et al.* (2021) also observed increased accumulation of glycine betaine in plants under water stress conditions.

Effect of water stress on super oxide dismutase enzyme (SOD)

Main responses of drought stress in plants are membrane lipid peroxidation and accumulation of reactive oxygen species (ROS). One of the key responses of drought stress is ROS scavenging. Antioxidant enzymes scavenge ROS molecules and thus protect

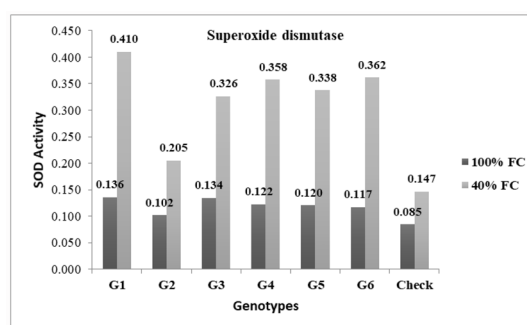


Fig. 1. Effect of drought stress on superoxide dismutase activity of cocoa genotypes.

* G1: P.IV 19.9; G2: P.II 12.11; G3: P.IV 59.8; G4: VSD.I 11.11; G5: VSD. I 3.4; G6: VSD.I 29.9; FC: Field capacity.

the cell membranes from oxidative damage. Increased activity of antioxidant enzymes indicates better drought tolerance under stress in plants (Carvalho *et al.* 2019). Super oxide dismutase is a part of complex antioxidant mechanism functions as a reactive oxygen species scavenger thus helps the plant to deal with water deficit stress. The obtained results pertaining to SOD activity in Fig. 1 depicts, significant variation in all the cocoa genotypes including check variety under both the levels of field capacity. The cocoa genotype, P.IV 19.9 (0.410 units/mg protein/g) which is categorized as highly drought tolerant recorded the higher SOD value and it was followed by the genotype VSD.I 29.9 (0.362 units/mg protein/g), VSD.I 11.11 (0.358 units/mg protein/g), VSD.I 3.4 (0.338 units/mg protein/g) and P.IV 59.8 (0.326 units/mg protein/g), respectively when compared to susceptible genotype P.II 12.11 (0.205 units/mg protein/g) and check variety (0.147 units/mg protein/g) which recorded lower SOD. Drought tolerant genotypes recorded higher values than susceptible at water stress condition. SOD value increased in 40% field capacity in all drought tolerant genotypes when compared to check variety which is highly susceptible indicates that SOD synthesis activated under water deficit conditions with complex antioxidant mechanism. Khayatnezhad *et al.* (2021) recorded significant variation in SOD activity and found that the higher SOD value for resistant varieties recorded when compared to susceptible in durum wheat genotypes under drought stress. They also stated that SOD activity can be used as a reliable index for drought stress tolerance. The similar line of studies were given by Xu *et al.* (2018) in soyabean,

Table 3. Correlation matrix among the drought tolerant contributing characters in cocoa genotypes.

	V1	PROLINE	NRA	SOD	GB
V1	1				
PROLINE	0.946**	1			
NRA	0.941**	0.972**	1		
SOD	0.942**	0.976**	0.914**	1	
GB	0.847**	0.842**	0.895**	0.788**	1

** Correlation significant at 0.01 level

V1 – Percentage of leaves retained

PROLINE – Proline ($\mu\text{g/g}$)

NRA – Nitrate reductase activity ($\text{mmol nitrate /g/hr}$)

SOD – Superoxide dismutase ($\text{units/mg protein/g}$)

GB – Glycine betaine ($\mu\text{ mol/g}$)

Wei *et al.* (2020) in safflower and Juby *et al.* (2021) in cocoa which reported higher SOD values in drought tolerant plants than susceptible one.

Correlation studies of biochemical parameters to drought stress

Correlation coefficients among the different physiological parameters with respect to percentage of leaves retained after stress period of one week were analyzed using Pearson's correlation and the results are presented in Table 3. When plants were subjected to drought stress, all biochemical indicators showed a positive and significant association with the percentage of leaves retained, showing that they play a key role in establishing drought tolerance mechanism among the, proline showed maximum correlation with the dependent variable, percentage of leaves retained (0.946). Proline has a direct correlation with drought adaptive capabilities of plants (Kishor and Sreenivasulu 2014, Bandruska *et al.* 2017). Among the other parameters, Nitrate reductase (0.941), glycine betaine (0.847) and superoxide dismutase (0.942) also expressed a significant and positive correlation with dependant variable. Due to the fact that the amount of NR enzyme normally declines under drought stress, hybrids with higher levels of NR enzyme was more resistant to drought stress and had a ability to control nitrogen uptake and protein synthesis (Juby *et al.* 2021). The results of NR enzyme was discovered to be directly associated to the dependent variable in the current study. Hence all the biochemical parameters studied had a highly significant correlation with re-

spect to dependent variable and these can be used as indicator for drought tolerance in cocoa genotypes.

CONCLUSION

In this investigation, the biochemical parameters such as proline and glycine betaine belongs to a group of osmolytes and another two parameters Nitrate reductase and superoxide dismutase belongs to enzyme group. All the genotypes marked under drought tolerant recorded higher proline content under stress condition than susceptible ones. All the genotypes under well watered condition at 100% FC showed least amount of proline content. Glycine betaine a composite osmolyte, also followed the similar pattern under water stress conditions. Drought tolerant genotypes were having higher amount of glycine betaine content than susceptible genotypes hence, these two biochemical parameters were accumulated and increased in levels when plants were subjected to drought stress than well watered condition. In the present study, drought tolerant plants exhibited higher activity of superoxide dismutase enzyme than susceptible genotypes under drought stress at 40% FC. But in case of Nitrate reductase it was reversed. Well watered plants at 100% FC recorded higher Nitrate reductase enzyme when compared to water deficit conditions (40% FC). Drought tolerant genotypes were having higher Nitrate reductase enzyme than check variety under stress condition which imparted them ability to withstand against stress effects. This study could help to understand the role of these biochemical parameters in drought adaptive mechanism of cocoa and these can be utilized as a most promising tool or indicators to screen the drought tolerance in cocoa genotypes.

REFERENCES

- Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 216d promotes growth and delays the onset of the drought response in *Theobroma cacao*. *J Exp Bot* 60 (11): 3279–3295.
- Bates L, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Pl Soil* 39: 205-207.
- Binimol B (2005) Identification of drought tolerant cocoa types. MSc (Agric) thesis. Kerala Agricultural University, Vellanikkara, Thrissur, pp 95.

- Carvalho M, Castro I, Moutinho-Pereira J, Correia C, Egea-Cortines M, Matos M, Rosa E, Carnide V, Lino-Neto T (2019) Evaluating stress responses in cowpea under drought stress. *J Pl Physiol*, pp 241-153001.
- Costa RCL, Lobato AKS, Oliveira Neto CF, Maia PSP, Alves GAR, Laughinghouse HD (2008) Biochemical and physiological responses in two *Vigna unguiculata* (L.) walp cultivars under water stress. *J Agron* 7: 98-101.
- Dhindsa RS, Plumb DP, Thorpe TA (1981) Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J Exp Bot* 32: 93-101.
- Din J, Khan SU, Ali I, Gurmani AR (2011). Physiological and agronomic response of canola varieties to drought stress. *J Anim Pl Sci* 21 (1): 78–82.
- Dutta P, Bandopadhyay P, Bera AK (2016) Din J, Khan SU, Ali I, Gurmani AR (2011) Physiological and agronomic response of identification of leaf based physiological markers for drought susceptibility during early seedling development of mung bean. *Am J Pl Sci* 7: 1921-1936.
- Dzandu E, Enu-Kwesi L, Markwei MC, Ayeh KO (2021) Screening for drought tolerance potential of nine cocoa (*Theobroma cacao* L.) genotypes from Ghana. *Heliyon* 7: e08389.
- Efeoglu B, Ekmeki Y, Cicek N (2009) Physiological responses of three maize cultivars to drought stress and recovery. *S Afr J Bot* 75(1): 34-42.
- Evans HJ, Nason A (1953) Pyridine nucleotide-nitrate reductase from extracts of higher plant. *Pl Physiol* 28: 233.
- Foyer CH, Valadier MH, Migge A, Becker TH (1998) Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. *Pl Physiol* 117: 283-292.
- Ghosh SC, Asanuma K, Kusutani K, Toyota M (2000) Effects of moisture stress at different growth stages on the amount of total non-structural carbohydrate, nitrate reductase activity and yield of potato. *Jpn J Trop Agric* 44: 158-166.
- Grieve CM, Grattan SR (1983) Rapid assay for determination of water-soluble quaternary amino compounds. *Pl Soil* 70: 303-307.
- International Cocoa Organization (ICCO) (2017) Quarterly bulletin of cocoa statistics 39(4): 24.
- Janani P, Kumar N, Jegadeeswari V (2019) Evaluation of cocoa (*Theobroma cacao* L.) clones under natural rainfed conditions for drought tolerance. *Chem Sci Rev Lett* 8 (32): 220-225.
- Juby B, Minimol JS, Suma B, Santhoshkumar AV, Jiji J, Panchami PS (2021) Drought mitigation in cocoa (*Theobroma cacao* L.) through developing tolerant hybrids. *BMC Pl Biol* 21:594.
- Kishor PBK, Sreenivasulu N (2014) Is proline accumulation *per se* correlated with stress tolerance or is proline homeostasis a more critical issue. *Pl Cell Environ* 37: 300-311.
- Li H, Lei P, Pang X, Li S, Xu H, Feng X (2017) Enhanced tolerance to salt stress in canola (*Brassica napus* L.) seedlings inoculated with the halotolerant *Enterobacter cloacae* HSNJ4. *Appl Soil Ecol* 119: 26–34.
- Mafakeri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohra bil Y (2010) Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars. *Aust J Crop Sci* 5(10): 1255-1260.
- Majeed S, Nawaz F, Naeem M, Yasin M, Ashraf MY, Ejaz S, Ahmad KS, Tauseef S, Farid G, Khalid I, Mehmood K (2020) Nitric oxide regulates water status and associated enzymatic pathways to inhibit nutrients imbalance in maize (*Zea mays* L.) under drought stress. *Pl Physiol Biochem* 155:147–160.
- More SJ, Kumari DS, Suresh Kumar J, Ravi V (2019) Water stress revealed physiological and biochemical variations in taro [*Colocasia esculenta* (L.) Schott] varieties/genotypes. *Int J Curr Microbiol Appl Sci* 8 (8): 2242-2253.
- Papageorgiou GC, Murata N (1995) The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving Photosystem II complex. *Photosynth Res* 44(3): 243-252.
- Plazas M, Nguyenb HT, Gonzalez-Orengac S, Fitaa A, Vicente O, Prohensa J, Boscaiu M (2019) Comparative analysis of the responses to water stress in eggplant (*Solanum melongena*) cultivars. *Pl Physiol Biochem* 143: 72–82
- Rhodes D, Hanson AD (1993) Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu Rev Pl Physiol Pl Mol Biol* 44(1): 357-384.
- Savaliya HB, Narwade AV, Zinzala NV, Faldu TA (2019) Effect of water stress on biochemical characteristics of summer mungbean (*Vigna radiata* (L.) Wilczek). *Int J Chem Studies* 7: 862-868.
- Souza CC, Oliveira FA, Silva IF, Amorim Neto MS (2000) Evaluation of methods of available water determination and irrigation management in “Terra Roxa” under cotton crop. *Rev Bras Engg Agric* 4: 338–342.
- Toscano S, Farieri E, Ferrante A, Romano D (2016) Physiological and biochemical responses in two ornamental shrubs to drought stress. *Front Pl Sci* 7:645.
- Voronin PV, Maevskaya SN, Nikolaeva MK (2019) Physiological and molecular responses of maize (*Zea mays* L.) plants to drought and rehydration. *Photosynthetica* 57 (3): 850–856.
- Wei B, Hou K, Zhang H, Wang X, Wua W (2020) Integrating transcriptomics and metabolomics to studies key metabolism, pathways and candidate genes associated with drought-tolerance in *Carthamus tinctorius* L. under drought stress. *Indust Crops Prod* 151: 12465.
- Xu C, Xia C, Xia Z, Zhou X, Huang J, Huang Z, Liu Y, Jiang Y, Casteel S, Zhang C (2018) Physiological and transcriptomic responses of reproductive stage soybean to drought stress. *Pl Cell Reports* 37:1611–1624.
- Xu ZZ, Yu ZW (2006) Nitrogen metabolism in flag leaf and grain of wheat in response to irrigation regimes. *J Pl Nutr Soil Sci* 169(1): 118-126.
- Yang F, Miao LF (2010) Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. *Silva Fenn* 44(1): 23-37.
- Zambrano MAO, Castillo DA, Perez LR, Teran W (2021) Cocoa (*Theobroma cacao* L.) response to water stress: Physiological characterization and antioxidant gene expression profiling in commercial clones. *Front Pl Sci* 12:700855.
- Zhang B, Chang L, Sun W, Ullah A, Yang X (2021) Overexpression of an expansin like gene, GhEXLB2 enhanced drought tolerance in cotton. *Pl Physiol. Biochem* 162: 468–472.